

AMENDMENTS TO THE CLAIMS

A listing of the claims presented in this patent application appears below. This listing replaces all prior versions and listing of claims in this patent application.

Claim 1 (previously amended): A method of detecting an extension reaction in which an extension reaction of a primer is detected, said method comprising the steps of:

(a) preparing a sample solution containing a nucleic acid, a primer having a base sequence that includes a complementary binding region which complementarily binds to said nucleic acid, and at least dATP or ddATP;

(b) allowing said sample solution to stand under a condition to cause said extension reaction, and producing pyrophosphate when said extension reaction is caused;

(c) bringing said sample solution into contact with the front face of a H^+ hardly permeable membrane having H^+ -pyrophosphatase, which penetrates from front to back of the membrane, of which active site that hydrolyzes pyrophosphate being exposed to the front face;

(d) measuring the H^+ concentration of at least either one of the solution at the front face side of said H^+ hardly permeable membrane or the solution at the back face side of said H^+ hardly permeable membrane, in a state where said H^+ -pyrophosphatase is immersed in the solution; and

(e) detecting said extension reaction on the basis of the result of measurement in the step (d).

Claim 2 (previously amended): A method of discriminating a base type in which the base type in a base sequence of a nucleic acid is discriminated, said method comprises the following steps of:

(a) preparing a sample solution containing a nucleic acid, a primer having a base sequence that includes a complementary binding region which complementarily binds to said nucleic acid, and at least dATP or ddATP;

(b) allowing said sample solution to stand under a condition to cause an extension reaction of said primer, and producing pyrophosphate when said extension reaction is caused;

(c) bringing said sample solution into contact with the front face of a H^+ hardly permeable membrane having H^+ -pyrophosphatase, which penetrates from front to back of the membrane, of which active site that hydrolyzes pyrophosphate being exposed to the front face;

(d) measuring the H^+ concentration of at least either one of the solution at the front face side of said H^+ hardly permeable membrane or the solution at the back face side of said H^+ hardly permeable membrane, in a state where said H^+ -pyrophosphatase is immersed in the solution;

(e) detecting said extension reaction on the basis of the result of measurement in the step (d); and

(f) discriminating the base type in the base sequence of said nucleic acid on the basis of the result of detection in the step (e).

Claim 3 (original): The method of discriminating a base type according to claim 2 wherein the difference between the H^+ concentration of the solution at said front face side, and the H^+ concentration of said sample solution post the step (b) and before the step (c) is measured, in the step (d).

Claim 4 (original): The method of discriminating a base type according to claim 3 wherein said extension reaction is detected by comparing the result of measurement in the step (d) with a control value, in the step (e).

Claim 5 (original): The method of discriminating a base type according to claim 4 wherein said discrimination of a base type is the discrimination of the base type of a SNP site, and

said control value is the result of measurement obtained in the step (d) through carrying out the steps (a), (b), (c) and (d) using a nucleic acid having said SNP site without mutation, as said nucleic acid.

Claim 6 (original): The method of discriminating a base type according to claim 2 wherein the H^+ concentration of the solution at said back face side is detected in the step (d), and said extension reaction is detected by comparing the result of measurement in the step (d) with a control value, in the step (e).

Claim 7 (previously amended): The method of discriminating a base type according to claim 6 wherein said discrimination of a base type is the discrimination of the base type of a SNP site,

said control value is the result of measurement obtained in the step (d) through carrying out the steps (a), (b), (c) and (d) using a nucleic acid having said SNP site with a different base type, as said nucleic acid.

Claim 8 (original): The method of discriminating a base type according to claim 2 wherein said H^+ concentration is optically measured in the step (d).

Claim 9 (original): The method of discriminating a base type according to claim 8 wherein a pH sensitive pigment or a membrane potential sensitive pigment is added to at least either one of the solution at said front face side and the solution at the back face side, in the step (d).

Claim 10 (previously amended): The method of discriminating a base type according to claim 9 wherein acridine orange or Oxonol V is added to at least either one of the solution at said front face side and the solution at the back face side, in the step (d).

Claim 11 (original): The method of discriminating a base type according to claim 2 wherein said H^+ concentration is electrically measured in the step (d).

Claim 12 (original): The method of discriminating a base type according to claim 2 wherein said extension reaction is an extension reaction according to a PCR method.

Claim 13-18 (canceled).

Claim 19 (previously amended): A method of detecting a nucleic acid having a particular base sequence, said method comprising the steps of:

(a) preparing a sample solution containing a sample, a primer having a base sequence that includes a complementary binding region which complementarily binds to said nucleic acid, and at least dATP or ddATP;

(b) allowing said sample solution to stand under a condition to cause an extension reaction of said primer, and producing pyrophosphate when said extension reaction is caused;

(c) bringing said sample solution into contact with the front face of a H^+ hardly permeable membrane having H^+ -pyrophosphatase, which penetrates from front to back of the membrane, of which active site that hydrolyzes pyrophosphate being exposed to the front face;

(d) measuring the H^+ concentration of at least either one of the solution at the front face side of said H^+ hardly permeable membrane or the solution at the back face side of said H^+ hardly permeable membrane, in a state where said H^+ -pyrophosphatase is immersed in the solution;

(e) detecting said extension reaction on the basis of the result of measurement in the step (d); and

(f) detecting the nucleic acid on the basis of the result of detection in the step (e).

Claim 20 (original): The method of detecting a nucleic acid according to claim 19 wherein the difference between the H^+ concentration of the solution at said front face side and the H^+ concentration of said sample solution post the step (b) and before the step (c) is measured in the step (d).

Claim 21 (original): The method of detecting a nucleic acid according to claim 20 wherein said extension reaction is detected by comparing the result of measurement in the step (d) with a control value, in the step (e).

Claim 22 (original): The method of detecting a nucleic acid according to claim 21 wherein said control value is the result of measurement obtained in the step (d) through carrying out the steps (a), (b), (c) and (d) using said sample without including a nucleic acid.

Claim 23 (original): The method of detecting a nucleic acid according to claim 19 wherein said H^+ concentration is optically measured in the step (d).

Claim 24 (original): The method of detecting a nucleic acid according to claim 23 wherein a pH sensitive pigment or a membrane potential sensitive pigment is added to at least either one of the solution at said front face side and the solution at said back face side, in the step (d).

Claim 25 (previously amended): The method of detecting a nucleic acid according to claim 24 wherein acridine orange or Oxonol V is added to at least either one of the solution at said front face side and the solution at said back face side, in the step (d).

Claim 26 (original): The method of detecting a nucleic acid according to claim 19 wherein said H^+ concentration is electrically measured in the step (d).

Claim 27 (original): The method of detecting a nucleic acid according to claim 19 wherein said extension reaction is an extension reaction according to a PCR method.

Claim 28-30 (canceled).

Claim 31 (new): The method of claim 1, wherein said H^+ hardly permeable membrane is prepared from vacuole isolated from cells.

Claim 32 (new): The method of claim 2, wherein said H^+ hardly permeable membrane is prepared from vacuole isolated from cells.

Claim 33 (new): The method of claim 19, wherein said H^+ hardly permeable membrane is prepared from vacuole isolated from cells.